CHEMICAL CHARACTERIZATION AND BIOLOGICAL STUDIES OF NEEM (*Azadirachta indica*) EXTRACTS

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ABSTRACT

Neem (*Azadirachta indica*) (Family: *Meliaceae*), also known as 'Pokok Mambu' in Malaysia is widely known to contain variety of bioactive compounds that had been proven for the cure of various infections and diseases related to toxicity and bacteria. The extractions of the plant constituents are mainly dependent on the extraction methods, conditions and solvents. This study aims on the extraction of the chemical constituents' and identification of the volatile constituents of *A. indica* extracts. The cytotoxicity effect was studied for the extracts that lead towards the isolation of a bioactive compound. The oil of the plant was studied for its physicochemical properties and antibacterial activity. The crude extracts (barks, leaves and roots) were extracted via solvent extraction (acetone, chloroform, maceration and refluxed in ethanol), while the fractions (hexane, chloroform, ethyl acetate and aqueous) were produced from partitioning of 80% methanol crude. Seed oil was extracted via Soxhlet with hexane for six hours. Volatile compound analysis via GC-MS was performed for all crude and fractions and cytotoxicity test against brine shrimp, *Artemia salina* for determination of LC_{50} after 24 h. The isolation and identification of bioactive compound from the most active fraction of cytotoxicity were performed via preparative-HPLC, UV-Vis, IR, MS and NMR. Physicochemical properties of oil were studied according to standard methods. Antibacterial activity of oil was determined against *B. subtilis, E. coli* and *S. aureus* via well diffusion method. Yields of the extracts were varied among different parts due to polarity of solvents and extraction conditions. Highest and lowest yields of crude extracts were leaf and bark reflux in ethanol with 5.46% and 0.13%, respectively. Minimum and maximum yield was obtained for fractions by root ethyl acetate (0.06%) and leaf chloroform (1.15%). The n-hexadecanoic acid was detected in all samples with seven similar compounds in both crude and fractions as the most abundant volatile compound. Cytotoxicity proves that root acetone (457.09 ± 0.88 ppm) and leaf ethyl acetate extract (1.35 ± 0.40 ppm) are the most toxic. All the fractions and only root acetone crude extract falls under toxic level (LC_{50} values < 500 ppm). The partitioning to different fractions separates the complex plant constituents according to polarity that influences the cytotoxicity. The isolation of quercetin-3-*O*-β-D-glucopyranoside from leaf ethyl acetate proves the cytotoxic effect. Major characteristics of the oils are; density: 0.95 g/cm^3; refractive index: 70.90; acid value: 4.80 mg KOH/g; free fatty acid (oleic): 4.75 %; iodine value: 93.09 $gI_2/100g$; pH: 4; peroxide value: 8.49 meq O_2/kg; moisture & volatile matter: 0.83 % and unsaponifiable matter: 1.84 %. The major fatty acid chains in the oil are; linoleic-: 34.69 %; oleic-: 20.46 %, stearic-: 20.42 % and palmitic acid: 18.66 %. Inhibition zone for antibacterial study with 20, 40, 60 and 80% of oil lies between 1.23 ± 0.03 to 1.70 cm, 1.33 ± 0.06 to 1.6 ± 1.57×10^{-16} cm and 1.4 ± 0.03 to 1.63 ± 0.03 cm for *B. subtilis*, *S. aureus* and *E. coli*, respectively. The MIC was 0.63± 0.0002, 2.50± 0.0010 and 5.00± 0.006% for *B. subtilis, E. coli* and *S. aureus,* respectively and *S. aureus* is more resistant. Results obtained supports that *A. indica* plant has high advantage to be used as drug in chemical and pharmaceutical industries. The study should be further continued through direct study with insect and human cell line to confirm the effect of the drug.

ABSTRAK

Neem (*Azadirachta indica*) (Keluarga: *Meliaceae*), dikenali sebagai Pokok Mambu di Malaysia mengandungi pelbagai juzuk kimia yang terbukti berkesan untuk mengubati pelbagai jenis penyakit berhubung dengan ketoksikan dan bakteria. Pengekstrakan juzuk kimia daripada tumbuhan bergantung pada cara dan situasi pengekestrakan serta pelarut kimia. Kajian ini bertujuan untuk mengekstrak juzuk kimia dan pengenalpastian juzuk kimia yang senang meruap daripada ekstrak *A. indica.* Fitokimia yang boleh meruap ditentukan dalam ekstrak pecahan kulit kayu, daun dan akar dan pengajian sitotoksiti menyumbang kepada pengasingan juzuk aktif. Ciri fisikokimia minyak dikaji mengikut kaedah piawai. Aktiviti anti-bakteria minyak ditentukan terhadap *B. subtilis, E. coli* dan *S. aureus* melalui kaedah penyebaran. Ekstrak mentah (kulit kayu, daun dan akar) diekstrak melalui pengekstrakan pelarut (aseton, kloroform, rendaman pada suhu bilik dan refluks dalam etanol), manakala ekstrak pecahan (heksana, kloroform, etil asetat dan air) dihasilkan daripada pembahagian sebanyak 80% ekstrak metanol mentah. Minyak biji diekstrak melalui soxhlet dengan heksana selama enam jam. Analisis sebatian meruap melalui GC-MS telah dilaksanakan ke atas semua ekstrak mentah dan pecahan dan ujian sitotoksiti terhadap udang air garam, *Artemia salina* melalui penentuan LC_{50} selepas 24 jam. Pengasingan dan pengenalpastian sebatian bioaktif daripada pecahan yang paling aktif sitotoksiti telah dijalankan melalui HPLC, UV-Vis, IR, MS dan NMR. Sifat-sifat fiziko-kimia dikaji mengikut kaedah piawai dan penilaian aktiviti anti-bakteria daripada minyak biji ke atas *B. subtilis, E. coli* and *S. aureus* dengan kaedah penyebaran. Hasil ekstrak berubah dalam kalangan bahagian yang berbeza kerana kekutuban pelarut dan syarat pengekstrakan. Hasil tertinggi dan terendah ekstrak mentah diperolehi daripada daun dan kulit kayu pengrefluksan etanol dengan 5.46% dan 0.13% masing-masing. Hasil minimum dan maksimum telah diperolehi bagi pecahan akar etil asetat (0.06%) dan daun kloroform (1.15%). Asid n-hexadecanoic dikesan dalam semua sampel dengan tujuh sebatian yang sama dalam kedua-dua mentah dan pecahan. Sitotoksiti membuktikan bahawa ekstrak aseton akar (457.09 ± 0.88 ppm) dan etil asetat daun (1.35 ± 0.40 ppm) adalah yang paling toksik. Semua pecahan dan hanya akar aseton ekstrak mentah dikategorikan dalam paras toksik (nilai LC_{50} <500 ppm). Pembahagian untuk pecahan yang berbeza memisahkan juzuk tumbuhan kompleks mengikut kekutuban yang mempengaruhi sitotoksiti. Pengasingan quercetin 3-*O*-β-D-glucopiranosida dari pecahan asetat etil daun menyokong data sitotoksiti. Ciri-ciri utama minyak adalah; ketumpatan: 0.95 g/cm^3; indeks biasan: 70.90; nilai asid: 4.80 mgKOH/g; asid lemak bebas (oleik): 4.75%; nilai iodin: 93.09 gI_2/100g; pH: 4; nilai peroksida: 8.49 $meqO_2$/kg; kelembapan & bahan meruap: 0.83% dan bahan tidak larut: 1.84 %. Rantai asid lemak utama dalam minyak ini adalah; asid linoleic: 34.69%; oleic: 20.46%, stearik: 20.42% dan palmitik: 18.66%. Zon perencatan untuk kajian antibakteria dengan 20, 40, 60 dan 80% daripada minyak terletak di antara 1.23 ± 0.03-1.70 cm, 1.33 ± 0.06-1.6 ± 1.57x10^{-16} cm dan 1.4 ± 0.03-1.63 ± 0.03 cm bagi *B. subtilis, S. aureus* dan *E. coli* masing-masing. MIC adalah 0.63 ± 0.0002, 2.50 ± 0.0010 dan 5.00 ± 0.006% bagi *B. subtilis, E. coli* dan *S. aureus* masing-masing dan *S. aureus* lebih menahan impak ekstrak. Keputusan yang diperolehi menyokong bahawa *A. indica* mempunyai kelebihan yang tinggi untuk digunakan sebagai dadah dalam industri kimia dan farmaseutikal. Kajian perlu diteruskan secara langsung dengan sel serangga dan manusia untuk mengesahkan kesan dadah.

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LIST OF SYMBOLS

-	Subtract
%	Percentage
~	Tilde
+	Add
<	Less-than
=	Equals
>	Greater-than
±	Plus-minus
≤	Less than or equal to
≥	More than or equal to
°C	Degree celsius
°C/min	Degree celsius per minute
µg/mL	Micrograms per milliliter
µL/mL	Microliter per milliliter
µm	Micrometer
a	Statistical alpha
bar	Unit of pressure
CFU/mL	Colony forming unit per mililiter
cm	Centimeter
eV	Electron volt
g	Gram
g/cm^3	Gram per cubic centimeter
g/g	Gram per gram

g/kg	Gram per kilogram
g/L	Gram per liter
g/mol	Gram per mol
GHz	Gigahertz
h	Hour
Hz	Hertz
KHz	Kilohertz
KV	Kilovolt
L	Liter
lbs	Pounds
Lx	Lux
m	Meter
M	Molarity
m/z	Mass-to-charge ratio
meq/kg	Milliequivalents per kilogram
mg	Miligram
mg/g	Miligram per gram
mg/kg	Miligram per kilogram
mg/mL	Miligram per mililiter
MHz	Megahertz
min	Minutes
mL	Mililiter
mL/kg	Mililiter per kilogram
mL/min	Mililiter per minute
mm	Milimeter

mTorr	Militorr
N	Normality
nm	Nanometer
ppm	Parts per million
ppt	Parts per trillion
psi	Pound-forceper square inch
s	Seconds
Tcm^{-2}	Torr per cubic centimeter
V	Volt
v/v	Volume per volume
W	Watt
w/v	Mass per volume ratio
wt%	Weight percentage
x	Multiple
δ	Delta
λ	Gamma
λ_{max}	Lamda maximum
μL	Microliter
π	Pi
π^*	Pi star
cm^{-1}	Reciprocal centimeter

LIST OF ABBREVATIONS

^{13}C-NMR	Carbon Nuclear Magnetic Resonance
1D-NMR	One dimensional Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
2D-NMR	Two dimensional Nuclear Magnetic Resonance
A. indica	*Azadirachta indica*
A. salina	*Artemia salina*
AOAC	Association of Analytical Chemists
AOCS	American Oil Chemists' Society
AED	Atom emission detector
AIDS	Acquired immunodeficiency syndrome
ANOVA	One way analysis of variance
ATCC	American type culture collection
B. subtilis	*Bacillus subtilis*
CO_2	Carbon dioxide
COSY	Correlated spectroscopy
DAD	Diode array detector
DEPT	Distortionless enhancement by polarization transfer
DMAE	Diffused microwave multi-mode cavity system
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
ECD	Electron capture detector
EI-MS	Electron Ionization-Mass Spectrometry
ELSD	Evaporate light scanning detector

FFA	Free fatty acid
FID	Flame ionization detector
FMAE	Focused microwave single-mode cavity system
FTIR	Fourier Transform Infrared
FTIRD	Fourier Transformed Infrared Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectroscopy
H	Hydrogen
HETCOR	Heteronuclear correlation spectroscopy
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HPLC	High Performance Liquid Chromatography
HUS	Hemolytic uremic syndrome
IR	Infrared
LC	Liquid chromatography
LD_{50}	Median lethal dose
LSD	Least significant difference
MAE	Microwave-assisted extraction
MH	Muller-Hinton
MIC	Minimum inhibitory concentration
MS	Mass Spectrometry
MSD	Mass selective detector
N	Nitrogen
NA	Nutrient agar
NIST	National Institute of Standards and Technology

NMR	Nuclear Magnetic Resonance
NPD	Nitrogen phosphorus detector
O	Oxygen
PV	Peroxide value
S	Sulfur
S. aureus	*Staphylococcus aureus*
SFE	Supercritical fluid extraction
Soxhlet	Solvent semi-continuous extraction
SPSS	Statistical Package for the Social Sciences
TCD	Thermal conductivity detector
TLC	Thin Layer Chromatography
TSA	Trypticase soy agar
TSP	Thermal separation probe
UNICEF	United Nations Children’s Fund
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet Spectroscopy
UV-A	Ultraviolet A
UV-B	Ultraviolet B
UV-C	Ultraviolet C
UV-Vis	Ultraviolet–Visible Spectroscopy
WHO	World Health Organization
XRC	X-ray crystallography

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

The aim of presenting this chapter was to present the motivation and problem statement, objectives and scope of the research together with the significance and contribution of the study. All these aspects would be a foundation in order to further discover the research.

1.2 BACKGROUND AND PROBLEM STATEMENT

Neem (*Azadirachta indica*) also known as ‘Pokok Mambu’ in Malaysia is an omnipotent tree that was classified under the mahogany family of *Meliaceae*. This plant had been used since prehistoric times as a traditional medicine for the treatment of chicken pox, fever, skin infections, oral care, as a tonic for ulcers, high blood pressure, diabetes, cholesterol and as hair care (Kumar and Navaratnam, 2013). The recent and modern usages of *A. indica* were also expanded to various fields such as for the replacement of petroleum with biodiesel, production of polyurethane coatings, as a modulator in rumen fermentation and the encapsulation of the seed oil for nano-emulsion (Ali et al., 2013 and Dhar et al., 2012). The native of *A. indica* plant was India and now the distribution in Malaysia is also wide. Several reported studies had been conducted on *A. indica* plant from Malaysia such as for the production of product for worm control, effect on c-Myc Oncogene expression in breast cancer cells and stimulation of Glucosamine (Chandrawathani et al., 2013; Kumar et al., 2011; Othman et al., 2011). Almost all the reported study in Malaysia was on the leaves extracts, but none of it includes the roots of the plant. Therefore, this present study reports the cytotoxic effect of *A. indica* bark, leaves and roots extracts specifically from Malaysia.

The modern (non-conventional) extraction techniques were being recently used for various studies, but in certain cases the traditional (conventional) methods were still effective according to the pros and cons reviewed in Table 2.2. Previous researchers, Carneiro et al. (2012), had reported that the extraction of ethanolic crude; and hexane, chloroform, dichloromethane and aqueous fractions of leaves of *A. indica* exhibit cytotoxic and antileishmanial activity in a single study. But, there were no any records on the comparison of both crude and fraction extracts on the bark and roots of *A. indica*. Therefore, a combined study of both techniques of crude and fractions was employed as the main motif in this single cytotoxic study under the same condition of the bioassay.

The extraction of plant metabolites includes the aid of the solvents (Azmir et al., 2013 and Gupta et al., 2012). The solvent polarity plays a role in producing extracts of different class of phytochemicals and resulted in various amounts of yield. High polar solvents were more prone in extracting high polar compounds and vice versa. Several scientific studies had been conducted for all three parts (bark, leaves and roots) of the plant with different solvents. But specifically for cytotoxic study of crude extracts of *A. indica* against brine shrimp, *Artemia salina*, only leaves and stem bark had been reported. Alcohols (methanol and ethanol) and aqueous cytotoxic study were reported for crude extract of the leaves and stem bark of the plant (Al-Emran, 2011 and Kirira et al., 2006). Whereas for the fractions; wide range of solvents had been reported such as aqueous, butanol, chloroform, ethyl acetate, hexane, methanol and petroleum ether on leaves (Al-Samarrai, 2013; Islam et al., 2012 and Kutama, 2008). Therefore, in this work, a combined effect of the crude acetone extract and fractions with other non-reported roots part were assayed against *A. salina* to evaluate the toxicity level.

The occurrence and the concentration of secondary metabolites in plants were restricted according to the plant taxonomy. Basically, these metabolites were not related to any primary metabolism, but they present to exhibit several biological activity or defense. Among these secondary metabolites, certain amounts were classified to be volatile compounds (Holopainen and Blande, 2012). As per the study conducted by

Hossain et al. (2013), the compounds present in *A. indica* was of different classes of hydrocarbon, terpenoids, phenolic, alkaloids, and their derivatives whereby certain were reported to reveal as biologically active molecules. The volatile compounds in *A. indica* leaves was reported for the butanol, chloroform, dichloromethane, ethyl acetate, hexane, methanol and petroleum ether extracts (Akpuaka et al., 2013; Helmy et al., 2007; Hossain et al., 2013; Moorthy and Boominathan, 2011 and Nand et al., 2012). One of the study reports the volatile compounds in bark extracts of dichloromethane, methanol and petroleum ether (Nand et al., 2012) and no study were reported for the roots extracts. Due to limited literature, this study has also focused on the volatile compound identification in both crude and fractions in order to report the possible activity of the plant and to support the cytotoxicity data.

One of the effective methods to evaluate the cytotoxicity of a substance was via shrimp lethality assay that was proposed since 1956 by Michael et al. The concept of this assay was to kill a laboratory-cultured invertebrate animal model, *A. salina*. This method was developed to predict the acute toxicity by avoiding the direct usage of laboratory animals that were being demanded by certain organizations and as these shrimp proven to correlate like a mice (Molina-Salinas et al., 2006). Moreover, it was recognized to be simple, fast, cheap, effective and reproducible for various assessments of toxicity for example in the detection of fungal and cyanobacteria toxins, natural products, pesticides and heavy metals (Carballo et al., 2002). This assay was vital in determining the responses of human normal and cancer cells as a preclinical assessment for drugs and was also widely used for *A. indica* extracts. In detail, the aim of this study also expands towards looking forward for bioactive compounds guided by the lethality bioassay of *A. salina*. Various compounds from *A. indica* had been reported to exhibit a cytotoxic effect, namely nimonol, nimbandiol, nimbolide, 2', 3'-dihydronimbolide and 28-deoxonimbolide (Takagi et al., 2014 and Wu et al., 2014). Therefore, isolation of a compound from the most cytotoxic extract of *A. indica* considered to be a scope of this work.

The seed oil of *A. indica* was another economic product since the oil has potential industrial uses and advantage in Indian medicine for many years (Gandhi et al., 1988). The fixed oil obtained from these seeds was the fatty oil that contains more fatty substances and denser compared to the essential oil that were volatile. Oils were a form of triglycerides that were non-polar and preferred to be extracted by non-polar solvent such as hexane (Ferreira-Dias et al., 2003). They were also different from extracts. Extracts were the crude mixtures as in the bark, leaves and roots of *A. indica*. The various fatty acids were reported to be present in *A. indica* seed oil with correlation of numerous biological activities as well as raw materials and feedstock's in industry (Atabani et al., 2013; Biswas et al., 2002; Dhar et al., 2012; Djenontin et al., 2012 and Kumar et al., 2010). However, other parameters on the physicochemical properties of the oil were also testified and proven to be influencing the choice of the industry, for example in the production of skin protector (Teressa et al., 2004) and biodiesel (Ali et al., 2013). The geographical variation, environmental condition and storage were being one of the key factors that impact the physicochemical properties of the plant oil (Dhar et al., 2012 and Goja, 2013). However, Malaysian *A. indica* oil has so far not been investigated to determine its physicochemical properties. This subsequently classifies the oil to suit world market requirements, especially if it is to be commercially produced in Malaysia as potential sources for export. Therefore, the soxhlet (solvent semi-continuous extraction) extraction of *A. indica* seed oil via hexane together with the study on the physicochemical and antibacterial properties would be one of the aims of this work to increase the economic feasibility for future commercial cultivation of this tree.

1.3 OBJECTIVES OF THE RESEARCH

The objectives of this present study were;

i. To investigate the different extraction techniques, conditions and solvents on the extraction of *A. indica* plant parts.
ii. To analyze and determine the volatile compounds of *A. indica* extracts.
iii. To study the cytotoxic effect of the extracts, isolate and identify compound from the most cytotoxic fraction of *A. indica*.
iv. To study the physicochemical and antibacterial properties of *A. indica* seed oil.

1.4 SCOPE OF THE RESEARCH

As a way to accomplish the objective of this study, the scope of this research focuses on the extraction of the crude of three different parts of *A. indica* by using three different solvents. Extractions for the solvent (ethanol) were performed at the different extraction condition of maceration and reflux. The same parts were also extracted with 80% of aqueous methanol and were further partitioned to four different fractions by using four different solvents. They were labelled as Fraction 1, 2, 3 and 4, respectively. Seed oil of *A. indica* was extracted via soxhlet method. All the extraction was conducted at room temperature except for soxhlet (mild heat) and reflux method at 60 °C.

The volatile compounds in the crude and fractions were analyzed and determined by performing chromatography and spectroscopy technique via Gas Chromatography-Mass Spectroscopy (GC-MS). All the injected liquid samples were prepared at similar concentration and solid samples at similar weight.

Brine shrimp larvae, *Artemia salina* was used to study the cytotoxic activities of both crude and fractions at different test concentrations. The results were analyzed to calculate the lethal concentrations of 50% of the sample population and the data's were evaluated in terms of probit analysis, further validated with Tukey's test and one way analysis of variance (ANOVA). Isolation of the compound was performed from the most cytotoxic fraction via preparative-High Performance Liquid-Chromatography (prep-HPLC) and the identification via absorption, functional groups, mass fragments and structure by using various spectroscopy methods.

The physicochemical properties of seed oil were studied according to standard methods proposed by American Oil Chemists' Society (AOCS), Association of Official Analytical Chemists (AOAC) and some reported literature. Besides that, to support the study, the seed oil alone was tested against three different pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*) to prove the antibacterial properties of the oil at four different concentrations (20, 40, 60 and 80%). In order to prove the effectiveness of the oil in fighting against the bacteria, the minimum inhibitory concentration of the oil was also determined through a broth tube dilution method. The data's were endorsed with Tukey's test and ANOVA.

1.5 SIGNIFICANCE AND CONTRIBUTION OF THE RESEARCH

One of the aims of the study was to differentiate the varying solvent polarity, ability and efficiency in producing the highest yield via different extraction techniques. The usage of different class of solvents in one similar study gives a better comparison on the extraction of the different plant parts that had never been studied before. Thus, the study points out the most suitable solvent and condition for the extraction of high yield *A. indica* extracts with complete utilization of all the major parts of the plant. The combined study of cytotoxicity of both crude and fractions of all plant parts in one study gives a better comparison on the toxic level presented by the plant.

The study of the plant roots of *A. indica* species from Malaysia for the first time and limited report concerning on the cytotoxic effect proves the capability of the plant growing in different geographical variation. The extract of the roots shows positive cytotoxic activity, whereby two of the roots crude extracts; acetone and chloroform extract present to be toxic (LC_{50} < 500 ppm) and less toxic ($\geq 500 \leq 1000$ ppm), respectively. Whereas all the fractions; hexane, chloroform, ethyl acetate and aqueous were toxic. Therefore, the present work on cytotoxicity was able to proof the activity of *A. indica* plant roots.

The volatile compound identification of roots acetone crude extracts presented in this study contributes towards a significant study since there was no existence of a previous report. The identified volatile compound of all the extracts gives a clearer image on the abundance of the bioactive compounds in each extract.

1.6 OVERVIEW OF THE THESIS

In detail, the Chapter 1 reveals the research motivation with the support of facts and figures from reporting studies. The objectives of the research were pointed out with an expended study scope. The brief significance or the contributions of the study were performed together with the necessity to contribute towards a research.

The Chapter 2 discusses the review of the literature or the background on the plant material *A. indica*, previous methods for the extraction, chromatography and spectroscopy techniques adapted to achieve the aim of the study. Several different methods were studied and were correlated with the current study. This method addresses some of the fundamental and practical methods that were suitable for the progress of the research.

As for Chapter 3, detail explanation of the material and methods were written according to the way of the analysis being conducted. The brief descriptions of the study were as drawn in the methodology flowchart whereby it represents the two different methods adapted.

Chapter 4 was allocated for results and discussion of the obtained data according to the objective whereby the yield of the extraction methods was discussed. The data in the analysis of the volatiles in the extracts were listed and reviewed according to different plant parts. The data on the cytotoxicity study were evaluated to provide scientific supporting data with the concentration level of sample that was required to behave as an aid in proving toxic effects. Lastly, the isolation and identification of a compound, quercetin-3-*O*-β-D-glucopyranoside was included from the extract that exhibits highest toxicity. The physicochemical and antibacterial studies of the seed oil reveal the capability of the plants grown it different geographical area.

The Chapter 5 represents the concluding chapter of the overall work and it summarizes the research work and point out the result in accordance with the objective of the study. Some recommendations were also suggested to expand the study in several terms.